

Application No. 10/663,999

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*AMENDMENTS TO THE CLAIMS*

This listing of claims replaces all prior versions, and listings, of claims in the application.

1-15. (Canceled).

16. (Currently Amended) A method for selectively separating live cells which have expressed a specific mRNA from a live cell group comprising:

a first step of determining a site of the specific mRNA that has high accessibility for oligonucleotide probe hybridization and preparing an oligonucleotide probe or probe set, labeled with a fluorescent dye or fluorescent dyes, having a base sequence or base sequences capable of hybridizing to the base sequence of the thus determined site, wherein the fluorescence of the fluorescent dye or fluorescent dyes will change upon the formation of a hybrid between the labeled oligonucleotide probe or probe set and the specific mRNA;

a second step of introducing the labeled oligonucleotide ~~probes~~ probe or probe set into cells in a live cell group containing the live cells which have expressed the specific mRNA and the live cells which have not expressed the specific mRNA, whereby the labeled oligonucleotide ~~probes~~ probe or probe set ~~hybridize~~ hybridizes to the specific mRNA expressed in the live cells;

a third step of irradiating light to the live cell group containing the live cells having hybridized and unhybridized oligonucleotide ~~probes~~ probe or probe set and the live cells having only unhybridized oligonucleotide ~~probes~~ probe or probe set and measuring the fluorescence which is emitted by the live cells, wherein the fluorescence from the cells having hybridized and unhybridized oligonucleotide ~~probes~~ probe or probe set is different from the fluorescence from the cells having only unhybridized oligonucleotide ~~probes~~ probe or probe set due to a change in fluorescence caused by hybrid formation, to identify the live cells wherein the hybrid formation of the labeled oligonucleotide ~~probes~~ probe or probe set and the specific mRNA has taken place; and

a fourth step of separating the identified live cells from the live cell group.

17. (Currently Amended) The method according to claim 16, wherein the probe set comprises a first probe and a second probe, the first probe and the second probe have base

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sequences ~~complementary to said mRNA and~~ capable of hybridizing to said mRNA adjacent to each other, thereto adjacently; and the first probe is labeled with an energy donor fluorescent dye and the second probe is labeled with an energy acceptor fluorescent dye, and the change in fluorescence is caused by fluorescence resonance energy transfer (FRET) from the energy donor fluorescent dye of the first probe to the energy acceptor fluorescent dye of the second probe.

18. (Previously Presented) The method according to claim 16, wherein the selective separation in the fourth step of the identified live cells based on the change in fluorescence is performed by a cell sorter.

19. (Previously Presented) The method according to claim 16, wherein the specific mRNA is a mRNA encoding a cytokine.

20. (Previously Presented) The method according to claim 16, wherein the live cells selectively separated in the fourth step are T Helper 1 (TH1) cells.

21. (Previously Presented) The method according to claim 16, wherein the live cells selectively separated in the fourth step are T Helper 2 (TH2) cells.

22. (Currently Amended) A method for selectively separating live cells which have expressed a mRNA encoding human interleukin-2 (IL-2) comprising:

a first step of introducing a probe ~~or probe set capable of labeling the mRNA~~ into cells in a live cell group containing live cells which have expressed the mRNA;

wherein the probe ~~or probe set comprises~~ has a base sequence ~~or base sequences~~ capable of specifically hybridizing to a ~~site of human IL-2 mRNA selected from the group consisting of the 287-316 site and the 342-371 site~~ and is labeled with a fluorescent dye or fluorescent dyes, wherein the fluorescence of the fluorescent dye ~~or fluorescent dyes~~ will change upon the formation of a hybrid between the labeled oligonucleotide probe ~~or probe set~~ and the mRNA; whereby the labeled oligonucleotide probe or probe set hybridizes to the specific mRNA expressed in the live cells;

a second step of irradiating light to the live cell group containing the live cells having hybridized and unhybridized oligonucleotide ~~probes~~ probe or probe set and the live cells

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having only unhybridized oligonucleotide ~~probes~~ probe or probe set and measuring the fluorescence which is emitted by the live cells, wherein the fluorescence from the cells having hybridized and unhybridized oligonucleotide ~~probes~~ probe or probe set is different from the fluorescence from the cells having only unhybridized oligonucleotide ~~probes~~ probe or probe set due to a change in fluorescence caused by hybrid formation, to identify the live cells wherein the hybrid formation of the labeled oligonucleotide ~~probes~~ probe or probe set and the mRNA has taken place; and

a ~~fourth~~ third step of selectively separating the identified live cells from the live cell group.

23-24. (Canceled).

25. (Currently Amended) The method according to claim 22, wherein the probe set comprises a first probe and a second probe, the first probe and the second probe have base sequences ~~complementary to the site and~~ capable of hybridizing to said mRNA adjacent to each other, thereto adjacently, and the first probe is labeled with an energy donor fluorescent dye and the second probe is labeled with an energy acceptor fluorescent dye, and said change in fluorescence is caused by fluorescence resonance energy transfer (FRET) from the energy donor fluorescent dye of the first probe to the energy acceptor fluorescent dye of the second probe.

26. (Currently Amended) A method for selectively separating live cells which have expressed a mRNA encoding human interleukin-4 (IL-4) comprising:

a first step of introducing a probe or probe set ~~capable of labeling the mRNA~~ into cells in a live cell group containing live cells which have expressed the mRNA;

wherein the probe or probe set ~~comprises~~ has a base sequence or base sequences capable of specifically hybridizing to a ~~site of~~ human IL-4 mRNA ~~selected from the group consisting of the 176-205 site and the 265-294 site~~ and is labeled with a fluorescent dye or fluorescent dyes, wherein the fluorescence of the fluorescent dye or fluorescent dyes will change upon the formation of a hybrid between the labeled oligonucleotide probe or probe set and the mRNA; whereby the labeled oligonucleotide probe or probe set hybridizes to the specific mRNA expressed in the live cells;

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a second step of irradiating light to the live cell group containing the live cells having hybridized and unhybridized oligonucleotide probes and the live cells having only unhybridized oligonucleotide probes and measuring the fluorescence which is emitted by the live cells, wherein the fluorescence from the cells having hybridized and unhybridized oligonucleotide probes is different from the fluorescence from the cells having only unhybridized oligonucleotide probes due to a change in fluorescence caused by hybrid formation, to identify the live cells wherein the hybrid formation of the labeled oligonucleotide probes and the mRNA has taken place; and

a ~~fourth~~ third step of selectively separating the identified live cells from the live cell group.

27-28. (Canceled).

29. (Currently Amended) The method according to claim 26, wherein the probe set comprises a first probe and a second probe, the first probe and the second probe have base sequences ~~complementary to the site and~~ capable of hybridizing to said mRNA adjacent to each other, thereto adjacently, and the first probe is labeled with an energy donor fluorescent dye and the second probe is labeled with an energy acceptor fluorescent dye, and said change in fluorescence is caused by fluorescence resonance energy transfer (FRET) from the energy donor fluorescent dye of the first probe to the energy acceptor fluorescent dye of the second probe.

30. (New) The method according to claim 22, wherein the probe or probe set is capable of hybridizing to the 287-316 site or the 342-371 site of human IL-2 mRNA.

31. (New) The method according to claim 25, wherein the probe set is capable of hybridizing to the 287-316 site or the 342-371 site of human IL-2 mRNA and includes a probe set corresponding to the SEQ ID NO: 7 and 8 or SEQ ID NO: 9 and 10.

32. (New) The method according to claim 26, wherein the probe or probe set is capable of hybridizing to the 176-205 site or the 265-294 site of human IL-4 mRNA.

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33. (New) The method according to claim 29, wherein the probe set is capable of hybridizing to the 176-205 site or the 265-294 site of human IL-4 mRNA and includes a probe set corresponding to the SEQ ID NO: 15 and 16 or SEQ ID NO: 17 and 18.